Int.J.Curr.Microbiol.App.Sci (2020) *9*(1): 1102-1119

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 1 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.901.125

Chemical Composition and Antibacterial Activity of Fractions from *Bridelia micrantha* Stem Bark Methanol Extract

Colette ElyseeAboudi Etono[1], Raymond Simplice Mouokeu[2], Guy SedarSingor Njateng[3], Alembert Tchinda Tiabou[4], Rebecca Madeleine Ebelle Etame[4], Rosalie Anne Ngono Ngane[1]* and MouelleSone Albert[5]

[1]Faculty of Sciences, University of Douala, P.O. Box 24157, Douala, Cameroon
[2]Institute of Fisheries and Aquatic Sciences, University of Douala, P.O. Box 7236, Douala, Cameroon
[3]Faculty of Sciences, University of Dschang, P.O. Box 67, Dschang, Cameroon
[4]Institute of Medical Research and Medicinal Plant Studies (IMPM), P.O. Box 6163, Yaoundé, Cameroon
[5]Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box 2701, Douala, Cameroon

**Corresponding author*

Keywords

Bridelia micrantha, Methanol extract, Fractionation, Antibacterial activity, Chromatography

Article Info

Accepted:
15 December 2019
Available Online:
20 January 2020

A B S T R A C T

Bridelia micrantha commonly known as coastal golden leaf is a member of the family *Phyllanthaceae*. In preliminary studies, nine fractions, named F_1- F_9, were obtained by fractionating the crude methanol extract of the stem bark of *Bridelia micrantha* using column chromatographic techniques. The F_6 fraction was found to be the most active when tested for the antibacterial activity. This study is thus aimed at investigating the effect of fractionation on antibacterial activity of F_6 fraction. The F_6 fraction was fractionated by adsorption chromatography on silica gel into eight sub-fractions designated F'_1- F'_8. A product was isolated from the dichloromethane/ methanol (10%) fraction and the structure was determined on the basis of spectroscopic data. The antibacterial activity of the F_6 fraction, sub-fractions and the product was evaluated by broth microdilution method against two reference strains and eighteen clinical bacterial strains. The chemical analysis of F_6 and three sub-fractions F'_3, F'_4 and F'_5 was done using HPLC-MS. The fraction F_6exhibited strong activity on all the tested bacteria with MIC values of 128 µg/ml on nine strains, including *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and *Salmonella sp*.The sub-fractions F'_4 and F'_5 exhibited the best activities on all the tested bacteria with MIC values of 32 to 256 µg/ml. The chemical analysis by HPLC-MS of F_6, F'_3, F'_4 and F'_5 revealed the presence of almost 180 identified compounds from various classes of secondary metabolites including alkaloids, flavonoids, steroids and terpenoids. The product obtained although inactive was elucidated as Daucosterol.

Introduction

The importance of medicinal plants in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the treatment of many intractable diseases (Umar *et al.*, 2018).Plant chemistry is the basis of the therapeutic uses of herbs. A good knowledge of the chemical composition of plants leads to a better understanding of its possible medicinal value (Hussein and El-Anssary, 2018). Plants produce a good deal of secondary metabolites that have variously been shown to exhibit interesting biological and pharmacological activities (Verpoorte, 1998). Secondary plant metabolites are classified according to their chemical structures into several classes. They are expected to form new sources of antimicrobial drugs, especially against bacteria (Namita and Mukesh, 2012). Antimicrobial resistance in bacterial pathogens is a worldwide challenge leading high morbidity and mortality in clinical settings (WHO, 2014). A selected group of bacteria described by the acronym of "ESCAPE" are the most frequent bacterial agents causing severe infections with significant MDR mechanisms. The term refers to *Enterococcus faecium*, *Staphylococcus aureus, Clostridium difficile*, *Acinetobacterbaumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* (covering all gram-negative enteric bacteria including *E. coli*, *K. pneumonia*, *Proteus spp.* and *Enterobacter spp*.) (Peterson, 2009).

A crude plant extract is a complex mixture in which compounds may interact antagonistically interfering with or masking the activity of one another(Nwodo*et al.*, 2010).One approach to solving this problem has been to separate the compounds to greater purity and to concentrate them into fractions by various processes, including by chromatography (Jean *et al.*, 2001). It is generally believed that fractionation of plant extracts and purification of the active principles would optimize their potencies. However, in some cases fractionation has been found to extend the spectrum of activity of plant extracts (Etame *et al.*, 2018; Etame *et al.*, 2019; Aboudi *et al.*, 2019), while in others it was found to reduce the spectrum of activity (Nwodo *et al.*, 2010), depending on whether certain constituents of the crude extract interact antagonistically, synergistically or additively when used in combination. Among the several medicinal plants distributed worldwide, *Bridelia micrantha* (*Phyllanthaceae*) is commonly used to treat several ailments including amoebic dysentery, cough, diarrhoea, gastric ulcer, eye diseases, infertility and tapeworms (Ngueyem *et al.*, 2009; Maroyi, 2017).

Preliminary studies from our research team highlighted the antibacterial activity of the stem bark methanol extract and a significant increase of this activity achieved with an active fraction F_6 following a partition of this methanol extract and column chromatography on silica gel of the dichloromethane (DCM) portion (Aboudi *et al.*, 2019). As a continuation to this previous work, the current study was initiated to investigate the effect of further fractionation of the active fraction F_6 of *B. micrantha* stem bark methanol extract on its antibacterial activity and to analyse its chemical composition.

Materials and Methods

Materials

Plant material

Fresh barks of *B. micrantha* used in this experiment were collected in January 2017 in the Centre Region of Cameroon at Mount

Kalla. The plant was identified at the Cameroon National Herbarium where a voucher specimen N° 5714 HNC (YA) was deposited.

Chemicals

Ciprofloxacin (Sigma-Aldrich, Germany) was used as reference antibiotic. *p*-Iodonitrotetrazolium chloride (Mouokeu*et al.,* 2014) was used as microbial growth indicator.

Microorganisms and Culture Media

The antibacterial activity was carried out on two reference strains (ATCC 27853, CIP 76110) and eighteen clinical strains. The clinical strains of *Escherichia coli* (EC 96, EC 99, EC 136, EC 137), *Enterobacter aerogenes* (ENT 119, ENT 144, ENT 167), *Klebsiella pneumonia* (KL 111), and *Staphylococcus aureus* (ST 9, ST 113, ST 120) were obtained from patient suffering from gastroenteritis at the Bafang ADLUCEM hospital. Those of *Salmonella enterica serovar typhi* (SAL 9), *Salmonella enterica serovar paratyphi B* (SPB), and *Salmonella enterica serovar typhimurium* (STM) were obtained from the Laboratory of Bacteriology and Mycology of the "Centre Pasteur" Yaounde-Cameroon.

Methicillin-resistant *Staphylococcus aureus* strains (MRSA 3, MRSA 9, MRSA 12) were obtained from the culture collection of the Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan. Multidrug resistant *Providencia stuartii* strain (PSNEA 16) was obtained from the culture collection of the University of Mediterranean, France. The characteristics of these bacteria were reported earlier (Aboudi *et al*., 2019).

Methods

Plant extraction

B. micrantha barks were collected and dried for 21 days in an ambient environment under shade and ground into powder. The powdered plant material (2.5 kg) was soaked in 10L of methanol for 3 days. The mixture was filtered using a Whatman N^{o}1 filter paper and the residue was re-extracted four times as previously described. The total methanol extract was concentrated using rotatory evaporator (Heidoph). The extract was further dried in an oven (VENTI-Line) at 45°C for 24 hours.

Fractionation of the crude extract

The extract (600 g) was dissolved in a mixture of methanol (2000mL), distilled water (1000mL) and dichloromethane (2000mL). The DCM phase and the methanol/water phase were separated. Each phase was concentrated using a rotatory evaporator. The DCM phase (150g) was chromatographed through a silica gel (250-300 Mesh) as described previously (Aboudi *et al.,* 2019). Nine fractions labeled F_1 to F_9 were obtained.

Fractionation of F_6 fraction

The F_6 fraction (24 g) was chromatographed through a silica gel (250-300 Mesh) column (2 cm internal diameter and 30 cm height) using DCM-MeOH (100:0; 95:5; 90:10; 80:20) as eluent. Seventy-three fractions of 150 mL each were collected and concentrated using rotary evaporator at 45°C under reduced pressure; then they were combined on the basis of their thin layer chromatography (TLC) profiles into eight major sub-fractions labelled F'_1 to F'_8 (F'_1: 1-7; F'_2: 8-15,17; F'_3: 18-25, F'_4: 26-35, F'_5: 36-47;F'_6: 48-59; F'_7: 60-69; F'_8: 70-73). Crystals were isolated

from the fraction 16 by recrystallizing with DCM/MeOH (v/v) followed by filtration. Ethyl acetate (100%) was used to wash crystals and revelation was done with UV (254-350 µm) first, then by using sulfuric acid 30%. The compound obtained was labeled CF_{16} (111mg).

Antibacterial activity assay

The *in vitro* antibacterial activity of the F_6 fraction, sub-fractions, and the purified compound was evaluated by determining the Minimum Inhibitory Concentrations (MIC) using broth microdilution method (CLSI, 2015). Briefly, the stock solution of F_6 fraction, sub-fractions, and the purified compound was prepared with 5% dimethylsulfoxide (DMSO) in broth culture medium. A bacterial suspension of about 1.5 x 10^8 CFU/ ml following N° 0.5 McFarland standard turbidity was prepared from an 18 hours old bacterial culture. These suspensions were further diluted in Mueller Hinton broth to give 1.5x 10^6 CFU/ml. The antibacterial susceptibility tests were performed in 96-well microtiter plates. A serial two-fold dilution of the F_6 fraction, sub-fractions was performed to obtain final concentrations ranging from 1024 to 8µg/ml in a total volume of 100 µl/well (the final concentrations of the purified compound were ranging from 256 to 2 µg/ml). These wells were finally inoculated with 100µl inoculum. The plates were incubated at 37°C for 18h. Following incubation, bacterial growth was monitored colorimetrically using *p*-iodonitrotetrazolium chloride (INT). Viable bacteria change the yellow dye of *p*-iodonitrotetrazolium violet into a pink colour. MIC value was recorded as the lowest concentration of the test substance that completely inhibited bacterial growth (Mouokeu *et al.*, 2014). The antibacterial activity was classified as *strong* if the extract displayed a MIC value less than 500 µg/ml, *moderate* from 500 to 1500 µg/ml and *weak* when over 1500 µg/ml (Aligiannis *et al.*, 2001).

The Minimum Bactericidal Concentrations (MBC) were determined by adding 50μl aliquots of the preparations which did not show any growth after incubation during MIC assays to 150 μL Mueller Hinton broth medium. These preparations were incubated at 37°C for 24h. The MBC values were regarded as the lowest concentration of extracts which did not produce any color change after addition of INT as mentioned above (Kuete *et al.*, 2009).

The experiments were performed in duplicate and repeated three times. Ciprofloxacin (Cipro) was used as positive control while 5% DMSO was used as negative control.

HPLC-MS analysis

HPLC was undertaken to assess the various components present in the fraction F_6 and sub-fractions F'_3, F'_4, F'_5. High resolution mass spectra were obtained with a Q-TOF Spectrometer (Bruker, Germany) equipped with a HESI source. The spectrometer was operated in positive mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using Na-Formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200°C. Nitrogen was used as sheath gas (10 L/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) HPLC system consisting of LC-pump, Diode Array Detector (DAD) (λ: 215, 254, 280, 330 nm), auto sampler (injection volume 5 µl) and column oven (50°C). The separations were performed using a synergic MAX-RP 100A (50x 2mm, 2.5µm particle size) with a H_2O (+0.1 % HCOOH) (A)/acetonitrile (+0.1 % HCOOH)

(B) gradient (flow rate 500 µL/min). Samples were analyzed using a gradient program as follows: 95 % A isocratic for 1.5 min, linear gradient to 100 % B over 6 min, after 100 % B isocratic for 2 min, the system returned to its initial condition (90 % A) within 1 min, and was equilibrated for 1 min.

Compound structural analysis

The chemical structure of CF_{16} was elucidated using spectroscopic data such as NMR 1D (^{1}H, ^{13}C, APT) and NMR 2D (COSY, HMBC). NMR^{13}C data were set using HMQC experiments while fragment arrangements were done using COSY.

Results and Discussion

The increasing prevalence of antimicrobial drug-resistant microorganisms recovered from hospitalized patients is a major concern worldwide (WHO, 2014). Many strains of *Staphylococcus aureus* and many strains of Gram negative bacteria display multi-drug resistance (GNPIN, 2018). Because of their safety and low cost as well as their impact on a large number of microbes, medicinal plants may have the ability to treat bacterial resistance to many types of antibiotics (Hassawi and Kharma, 2006).The antimicrobial effects of extracts from a large number of plants have been evaluated and reviewed (Mouokeu *et al.*, 2011, Ngono *et al.*, 2011) and the mechanisms that enable the natural ingredients of herbs to resist microbes have been discussed (Montanari *et al.*, 2012;Etame *et al.*, 2018). The results show that these mechanisms vary greatly depending on the components of the extract (Holley and Patel, 2005); that can actually be concentrated by the means of fractionation for optimal activity.

The F_6 fraction from methanol extract of *B. micrantha* stem bark, its sub-fractions, and compound were evaluated for their antibacterial activities on a panel of bacteria strains including two reference strains and eighteen clinical strains (Table 1). These results showed strong activity of the F_6 fraction on all the tested bacteria with MIC values of 128 µg/ml on nine strains, including *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus, Enterobacter aerogenes, and Salmonella sp* strains.

Previous authors reported the antibacterial activity of this plant **(**Steenkamp *et al.,* 2007; Gangoué-Piéboji *et al.,* 2009; Adefuye *et al.,* 2011). Adefuye *et al.,* (2011) revealed MIC_{50} values of ethyl acetate and acetone stem bark extract of *B. micrantha* ranged from 78 to 1250µg/ml and 78 to 625µg/ml respectively on *Staphylococcus aureus, Shigellasonnei, Salmonella Typhimurium,* and *Helicobacter pylori* strains.Gangoué-Piéboji*et al.,* (2009) evaluated the antibacterial activities of *B. micrantha* methanol stem bark extract against *E. coli, P. aeruginosa,* and *S. aureus* by using agar-dilution assay. The MIC values of methanol extract against the tested bacteria were of 1250µg/mL. Steenkamp *et al.,* (2007) using broth micro-dilution method found that the methanol bark extract showed MIC value of 4000µg/mL against *S. aureus*. These results line up with those obtained in this work, and point out the *B. micrantha* barks as a source of antibacterial compounds.

After the fractionation process of the F_6 fraction, eight sub-fractions were obtained. Among them, two were (F'_1 and F'_2) found to be inactive on all the tested bacteria; while the six others (F'_3,F'_4,F'_5,F'_6, F'_7, F'_8)exhibited antibacterial activity with MIC values ranging from 32 to 1024µg/ml. F'_3showed strong activity on fifteen tested bacteria strains with MIC value of 64 to 256 µg/ml.F'_4 and F'_5 were the most active considering their MIC value. They showed strong activity on all the twenty tested bacteria strains with MIC value

of 64 to 256 µg/ml for F'$_4$,while MIC values of 32 to 256 µg/ml were obtained with F'$_5$.This latter was found to be more efficient on *S. aureus* sensitive clinical strains (ST9, ST113, and ST120) with MIC value of 32 µg/ml (Table1). Thus from the fraction F_6 to sub-fractions F'$_3$, F'$_4$ and F'$_5$ the antibacterial activity increases. Increase antibacterial activity with fractionation reveals that the active principles of the stem barks of this plant are concentrated during fractionation in some fractions and highlights the fractionation as alternative to ameliorate plant extracts antimicrobial activity. Similar approach was reported by several authors (Khan *et al.,* 2011; Adefuye and Ndip, 2013; Etame*et al.,* 2019).

The sub-fractions F'$_4$ and F'$_5$were found to be the most active with this activity extended to methicillin-resistant *Staphylococcus aureus* (MRSA strains). Infections caused by MRSA are generally severe with the highest mortality rate (Cosgrove *et al.,* 2003). Actually very few antibiotics as Lysocin E are efficient against MRSA (Hamamoto *et al.,* 2015). The activity of these sub-fractions was extended to ESCAPE pathogens, particularly *S. aureus, K. pneumonia, P. aeruginosa, E. aerogenes* strains. The ESCAPE pathogens are differentiated from other pathogens due to their increased resistance to commonly used antibiotics. This increased resistance, combined with their clinical significance in the medical field, results in a necessity to combat them with novel antibiotics (Terra *et al.,* 2018). Therefore, the sub-fractions F'$_4$ and F'$_5$could be used directly as antibacterial or could provide molecules which could be useful as antibacterial or substrates for the synthesis of new broad spectrum antibiotics to overcome gastrointestinal tract infection bacteria.

Regarding the MBC values of the fractions F_6 to sub-fractions F'$_3$, F'$_4$ and F'$_5$, it was seen that MBC/MIC ratio was less than 4 in many cases meaning that they all exerted a bactericidal activity on many of the tested organisms (Marmonier, 1990; Djeussi *et al.,* 2013).

Figure 1

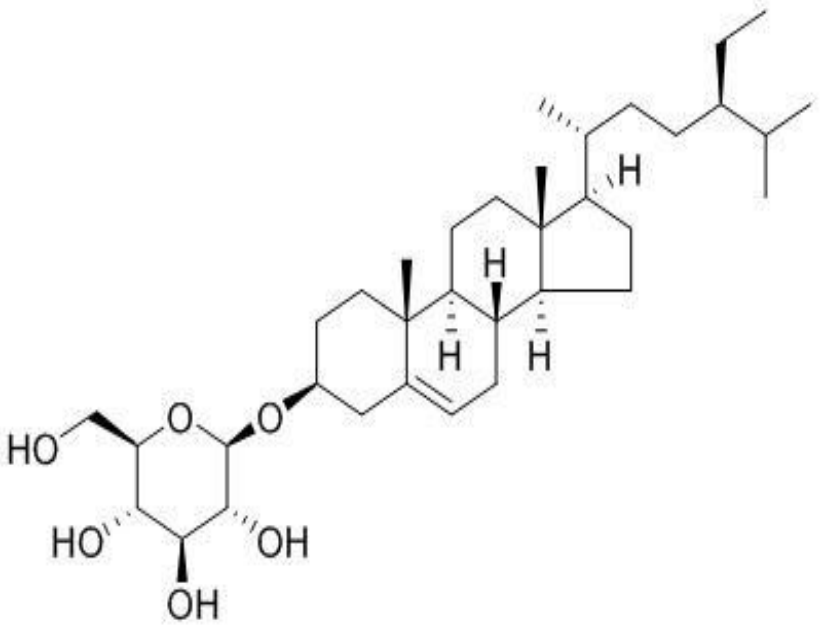

Table.1 MIC/MBC of F_6 fraction and sub-fractions from the methanol extract of *B. micrantha* stem bark (µg/mL)

Bacteria	F_6		R	F'_3	R	F'_4	R	F'_5	R	F'_6	R	F'_7	R	F'_8	R		Cipro	R
K. pneumoniae																		
KL111	256/256		1	512/512	1	256/512	2	256/256	1	512/1024	2	512/1024	2	512/1024	2		1/16	16
E. aerogenes																		
ENT167	128/512		4	512/1024	2	128/1024	8	128/1024	8	512/-	-	512/-	-	512/-	-		8/128	16
ENT144	256/256		1	512/512	1	256/512	2	128/512	4	256/1024	4	512/1024	2	512/1024	2		1/-	-
ENT119	256/512		2	256/512	2	128/256	2	64/512	8	512/1024	2	256/1024	4	512/512	1		1/-	-
S. aureus																		
ST9	128/512		4	128/512	4	128/256	2	32/256	8	128/1024	8	256/1024	4	256/1024	4		1/64	64
ST113	128/512		4	128/512	4	128/512	4	32/512	16	128/-	-	128/1024	8	128/-	-		1/64	64
ST120	256/512		2	128/512	4	64/512	8	32/256	8	256/1024	4	512/1024	2	512/1024	2		8/128	16
MRSA9	256/256		1	256/512	2	128/512	4	128/256	2	512/-	-	256/1024	4	256/-	-		2/4	2
MRSA3	128/256		2	256/1024	4	256/512	2	256/256	1	512/-	-	512/-	-	512/-	-		32/-	-
MRSA12	256/512		2	256/1024	4	128/512	4	128/512	4	512/1024	2	1024/-	-	1024/-	-		2/16	8
E. coli																		
EC96	128/512		4	256/1024	4	128/1024	8	64/1024	16	512/-	-	512/-	-	512/-	-		1/128	128
EC99	256/-		-	128/512	4	128/512	4	128/512	4	256/1024	4	256/1024	4	256/1024	4		4/16	4
EC136	256/256		1	256/512	2	128/512	4	64/512	8	512/1024	2	256/1024	4	256/1024	4		1/8	8
EC137	128/256		2	256/512	2	128/256	2	64/128	2	256/1024	4	512/1024	2	512/1024	2		16/128	8
P. stuartii																		
PSNEA16	256/-		-	512/512	1	128/512	4	128/512	4	512/1024	2	256/1024	4	256/1024	4		1/16	16
S. Typhi																		
SAL 9	256/-		-	256/512	2	128/512	4	256/512	2	256/1024	4	128/1024	8	256/1024	4		1/16	16
S. typhimurium	128/512		4	64/1024	16	64/128	8	128/512	4	256/-	-	128/-	-	128/-	-		32/64	2
S. paratyphi B	128/512		4	128/512	4	64/1024	16	64/512	8	512/1024	2	1024/1024	1	512/1024	2		1/128	128
P. aeruginosa																		
ATCC 27853	256/512		2	512/512	1	256/512	2	256/256	1	512/1024	2	512/1024	2	512/-	-		1/64	64
CIP 76110	128/512		4	64/512	8	64/512	8	128/256	2	256/1024	4	256/1024	4	128/1024	8		1/64	64

- = MIC or MBC that was greater than 1024

Table.2 Isolated compounds by the HPLC-MS

Compound name	Chemical formula	F_6 Fraction	F'_3 Fraction	F'_4 Fraction	F'_5 Fraction	Compound Class
Sacranoside A	$C_{21}H_{34}O_{10}$	✓	x	x	x	
Verbenol	$C_{10}H_{16}O$	✓	x	x	x	
p-Cymene	$C_{10}H_{14}$	✓	x	x	x	
Schizonepetoside C	$C_{16}H_{26}O_7$	✓	x	x	x	
Neohancoside B	$C_{21}H_{36}O_{11}$	✓	x	x	x	
Neohancoside A	$C_{21}H_{36}O_{10}$	✓	x	x	x	
Thujopsadiene	$C_{15}H_{22}$	✓	x	x	x	
Widdrol	$C_{15}H_{26}O$	✓	x	x	✓	
Dendroside E	$C_{21}H_{36}O_8$	✓	x	x	x	
Dendroside E	$C_{21}H_{36}O_8$	✓	x	x	x	
Trilobolide	$C_{27}H_{38}O_{10}$	✓	x	x	x	
Pterosin E	$C_{14}H_{16}O_3$	✓	x	x	x	
Ursiniolide A	$C_{22}H_{28}O_7$	✓	x	x	x	
5alpha-Acetyl-5alpha-decinnamoyltaxagifine	$C_{30}H_{40}O_{13}$	✓	x	x	x	
Homofukinolide	$C_{25}H_{34}O_6$	✓	x	x	x	
Vernodalin	$C_{19}H_{20}O_7$	x	x	x	✓	
Roseoside	$C_{19}H_{30}O_8$	✓	x	x	x	
Valerenicacid	C15 H22 O2	x	x	x	✓	
Turmerone	$C_{15}H_{20}O$	✓	x	x	x	
Marioside	$C_{22}H_{34}O_{10}$	✓	x	x	x	
Psilostachyin	$C_{15}H_{20}O_5$	x	✓	✓	✓	
Oriediterpenol	$C_{20}H_{32}O_2$	✓	✓	x	✓	
2,5,7-Trihydroxy-6,8-dimethyl-3-(4'-methoxybenzyl)chroman-4-one	$C_{19}H_{20}O_6$	✓	x	x	x	
Taxezopidine B	$C_{26}H_{38}O_{10}$	✓	x	x	x	

Taxuspine W	$C_{26}H_{36}O_9$	✓	x	x	x	**Terpenoids**
Taxuyunnanine E	$C_{33}H_{42}O_{12}$	✓	x	x	x	
Taxumairol B	$C_{28}H_{40}O_{12}$	✓	x	x	x	
Shikokianin	$C_{24}H_{32}O_8$	✓	x	x	x	
Taxumairol C	$C_{28}H_{38}O_{11}$	✓	x	x	x	
Lungshengenin G	$C_{26}H_{34}O_9$	✓	x	x	x	
Yadanzioside M	$C_{33}H_{40}O_{15}$	✓	x	x	x	
10-Hydroxyacetylbaccatin VI	$C_{37}H_{46}O_{15}$	✓	x	x	x	
Taxuspine U	$C_{28}H_{40}O_{11}$	✓	x	x	x	
Baccatin VI	$C_{37}H_{46}O_{14}$	✓	x	x	x	
Taxchin B	$C_{41}H_{52}O_{14}$	✓	x	x	x	
9(betaH)-9-Dihydro-19-acetoxy-10-deacetylbaccatin III	$C_{31}H_{40}O_{12}$	✓	x	x	x	
13-Deacetoxy-13,15-epoxy-11(15-->1)-abeo-13-epi-baccatin VI	$C_{35}H_{42}O_{12}$	✓	x	x	x	
cis-Neoabienol	$C_{20}H_{34}O$	✓	x	x	✓	
9-Deacetyl-9-benzoyl-10-debenzoyltaxchinin A	$C_{31}H_{40}O_{10}$	✓	x	x	x	
Taxuspine O	$C_{26}H_{36}O_{10}$	✓	x	x	x	
Forskoditerpenoside C	$C_{28}H_{44}O_{11}$	✓	x	x	x	
Ganolactone	$C_{27}H_{36}O_6$	x	x	x	✓	
3beta-Acetyl ursa-14-en-16-one	$C_{32}H_{50}O_3$	x	x	x	✓	
Nigakilactone I	$C_{21}H_{28}O_6$	x	x	✓	✓	
Lup-20(29)-ene-3alpha-acetoxy-24-oic acid	$C_{32}H_{50}O_4$	x	✓	x	✓	
3-Hydroxy-25-norfriedel-3,1(10)-dien-2-one-30-oic acid	$C_{29}H_{42}O_4$	x	✓	x	✓	
Camellin	$C_{18}H_{30}O_7$	✓	x	x	x	
Sobrerol	$C_{10}H_{18}O_2$	✓	✓	x	✓	
Hypolidemethylether	$C_{21}H_{26}O_3$	✓	x	x	x	
Isovaleroxy-hydroxy dihydrovaltrate	$C_{27}H_{40}O_{11}$	✓	x	x	x	

Taxuspine F	$C_{28}H_{38}O_{10}$	✓	✓	x	✓	
Valerosidatum	$C_{21}H_{34}O_{11}$	✓	x	x	x	
8-(O-Methyl-p-coumaroyl)harpaside	$C_{25}H_{32}O_{12}$	✓	x	x	x	
Riddelline	$C_{18}H_{23}NO_6$	✓	x	x	x	**Alkaloids**
Serratinidine	$C_{18}H_{28}N_2O_2$	✓	x	✓	✓	
Securinol C	$C_{13}H_{17}NO_3$	✓	x	x	x	
Argentine	$C_{23}H_{26}N_4O_3$	✓	x	x	x	
Ervadivaricatine A	$C_{43}H_{56}N_4O_5$	x	x	x	✓	
1beta,2beta,5alpha,11-Tetraacetoxy-8alpha-benzoyl-4alpha-hydroxy-7beta-nicotinoyl-dihydroagarofuran	$C_{36}H_{41}NO_{14}$	x	x	x	✓	
3-O-Tetradecanoyl-1-cyano-2-methyl-1,2-propene	$C_{19}H_{33}NO_2$	✓	✓	x	✓	
Wilsonine	$C_{20}H_{25}NO_4$	x	x	x	✓	
Nor-orixine	$C_{16}H_{19}NO_6$	✓	x	x	x	
Chelirubine	$C_{21}H_{16}NO_5$	x	✓	x	✓	
Dihydrokoumine	$C_{20}H_{24}N_2O$	x	x	x	✓	
N-Methyltyramine-O-alpha-L-rhamnopyranoside	$C_{15}H_{23}NO_5$	✓	x	x	x	
Pseudobrucine	$C_{23}H_{26}N_2O_5$	✓	x	x	x	
Euoverrine A	$C_{48}H_{51}NO_{18}$	✓	✓	✓	✓	
Subaphyllin	$C_{14}H_{20}N_2O_3$	✓	x	x	x	
Aldohypaconitine	$C_{33}H_{43}NO_{11}$	✓	x	x	x	
Ergocornine	$C_{31}H_{39}N_5O_5$	✓	x	x	x	
Geniculine	$C_{34}H_{47}NO_{11}$	✓	x	x	x	
Teixidol	$C_{28}H_{40}O_{10}$	✓	x	x	x	
Thalicmine	$C_{21}H_{23}NO_5$	✓	x	x	✓	
Lysergamide	$C_{16}H_{17}N_3O$	✓	x	x	✓	

12-Methoxyaffinisine	$C_{21}H_{26}N_2O_2$	✓	x	x	x	
Voacamine	$C_{43}H_{52}N_4O_5$	✓	x	x	x	
Terminaline	$C_{23}H_{41}NO_2$	✓	✓	x	x	
Camptothecin	$C_{20}H_{16}N_2O_4$	✓	✓	x	✓	
11-Deoxojervine	$C_{27}H_{41}NO_2$	✓	✓	x	x	
Germerine	$C_{37}H_{59}NO_{11}$	✓	✓	x	x	
Buxbodine D	$C_{28}H_{46}N_2O$	✓	x	x	x	
Parasorbicacid	$C_6H_8O_2$	✓	✓	x	✓	**Phenolic compounds**
Danshensu	$C_6H_{10}O_5$	✓	✓	x	✓	
2-Hexenyl benzoate	$C_{13}H_{16}O_2$	x	x	x	✓	
Phenethylcaffeate	$C_{17}H_{16}O_4$	✓	x	x	x	
6'-O-Methylhonokiol	$C_{19}H_{20}O_2$	✓	x	x	✓	
Protocatechuoylcalleryanin	$C_{20}H_{22}O_{11}$	✓	✓	x	✓	
6-Shogaol	$C_{17}H_{24}O_3$	x	x	x	✓	
4-Prenyl dihgdropinosylvin	$C_{19}H_{22}O_2$	x	✓	✓	✓	
Thelephantin C	$C_{32}H_{30}O_9$	x	✓	x	✓	
Salicylic acid	$C_7H_6O_3$	x	✓	x	✓	
Tropolone	$C_7H_6O_2$	x	✓	x	✓	
Vanillyl alcohol	$C_8H_{10}O_3$	x	✓	x	✓	
Pyrogallol	$C_6H_6O_3$	✓	x	x	x	
Phenyl-2-propanone	$C_9H_{10}O$	x	✓	✓	✓	
alpha-Thujaplicin	$C_{10}H_{12}O_2$	x	x	x	✓	
9,12-Dihydroxy-15-nonadecenoic acid	$C_{19}H_{36}O_4$	✓	x	✓	✓	
Palmitoleicacid	$C_{16}H_{30}O_2$	✓	✓	x	✓	
Valerenolicacid	$C_{16}H_{24}O_2$	✓	x	✓	✓	
9,10-Dihydroxystearic acid	$C_{18}H_{36}O_4$	✓	✓	x	✓	
Methyl 9-octadecenoate	$C_{19}H_{36}O_2$	✓	✓	x	✓	
Trichosanicacid	$C_{18}H_{30}O_2$	✓	✓	✓	✓	
Hydnocarpicacid	$C_{16}H_{28}O_2$	x	✓	x	✓	
Ethyloctadecanoate	$C_{20}H_{40}O_2$	x	✓	x	✓	

Nilicacid	$C_5 H_{10} O_3$	x	x	x	✓	**Fatty acids**
4,8,12-Trimethyl tridecanoic acid	$C_{16} H_{32} O_2$	x	✓	x	✓	
Coronaricacid	$C_{18} H_{32} O_3$	x	x	x	✓	
Docosandioicacid	$C_{22} H_{42} O_4$	x	x	x	✓	
Tetradecenoicacid C	$C_{14} H_{26} O_2$	x	✓	x	✓	
Gadoleicacid	$C_{20} H_{38} O_2$	x	x	x	✓	
Eucalyptus wax	$C_{33} H_{64} O_2$	✓	x	x	x	
Sarcostin	$C_{21} H_{34} O_6$	✓	x	x	x	**Steroids**
Strophanthidin	$C_{23} H_{32} O_6$	✓	x	x	x	
Bufotalin	$C_{25} H_{34} O_7$	✓	x	x	x	
Taccalonolide H	$C_{36} H_{44} O_{14}$	✓	x	x	x	
β- sitosterol 3-O- β- D-glucopyranoside	$C_{35} H_{60} O_6$	✓	x	x	x	
Cinobufagin	$C_{26} H_{34} O_6$	✓	✓	✓	✓	
1,4-Epoxy-16-hydroxyheneicos-1,3,12,14-tetraene	$C_{21} H_{34} O_2$	✓	✓	x	x	
5beta-Cholanic acid	$C_{24} H_{40} O_2$	✓	✓	x	✓	
Sengosterone	$C_{29} H_{44} O_9$	✓	x	x	x	
4-Methyl-7-ergosta-8,24(28)-diene	$C_{29} H_{48}$	✓	✓	x	x	
Stigmasta-4,25-dien-3beta,6beta-diol	$C_{29} H_{48} O_2$	✓	x	x	x	
25R-Spirost-4-en-3,12-dione	$C_{27} H_{38} O_4$	x	x	x	✓	
Flavaspidinin	$C_{23} H_{30} O_8$	✓	x	x	x	**Flavonoids**
3,5-Diacetyltambulin	$C_{22} H_{20} O_9$	x	✓	✓	✓	
Retusine	$C_{16} H_{25} N O_5$	✓	x	x	x	
Agastachin	$C_{47} H_{44} O_{22}$	✓	✓	x	x	
Triacetylhispidulin	$C_{22} H_{18} O_9$	x	x	x	✓	
Ergochrysin	$C_{31} H_{28} O_{14}$	x	x	x	✓	
Mulberrofuran Q	$C_{34} H_{24} O_{10}$	x	✓	x	✓	
Theasinensin A	$C_{44} H_{34} O_{22}$	x	x	x	✓	

Methyl-3-O-beta-D-glucopyranosyl polygalacate	C_{37} H_{60} O_{11}	x	x	x	✓	**Glucosides**
Coelovirin A	C_{21} H_{30} O_{12}	✓	x	x	x	
1,1'-Dibenzene-6',8',9'-trihydroxy-3-allyl-4-O-beta-D-glucopyranoside	C_{24} H_{30} O_{9}	✓	x	x	x	
Tetracentronside B	C_{26} H_{32} O_{11}	✓	x	x	x	
Magnoshinin	C_{24} H_{30} O_{6}	✓	x	✓	✓	
Sterekunthal B	C_{20} H_{18} O_{4}	✓	x	x	x	**Phtalide derivatives**
Theaspirone	C_{13} H_{20} O_{2}	✓	x	x	✓	
Senkyunolide K	C_{12} H_{16} O_{3}	✓	✓	x	x	
Senkyunolide M	C_{16} H_{22} O_{4}	x	✓	x	✓	
Adenine	C_{5} H_{5} N_{5}	✓	✓	x	x	**Purines**
Hypoxanthine	C_{5} H_{4} N_{4} O	x	x	x	✓	
Zeatin	C_{10} H_{13} N_{5} O	x	x	x	✓	
Prenylcaffeate	C_{14} H_{16} O_{4}	✓	x	x	x	**Coumarins**
6-Hydroxy-7-methylesculetin	C_{10} H_{8} O_{3}	✓	x	x	x	
Quassimarin	C_{26} H_{34} O_{11}	✓	✓	x	x	**Quassinoids**
Picrasinoside C	C_{28} H_{42} O_{11}	✓	x	x	x	
alpha:beta-Diolein	C39 H72 O5	x	✓	x	✓	**Diglycerides**
Glyceride-1,3-dipalmito-2-sorbate	C41 H74 O5	x	✓	x	✓	
Phycocyanobiline	**C_{33} H_{36} N_{4} O_{6}**	✓	**x**	**x**	**x**	**Bile pigments**
Biliverdin	C_{33} H_{34} N_{4} O_{6}	✓	x	x	x	
3-Methylcyclotridecan-1-one	C_{14} H*26* O	x	✓	x	✓	**Ketone**
Civetone	C_{17} H_{30} O	x	x	x	✓	
Maesaquinone	C26 H42 O4	✓	x	x	x	**Quinones**
Methylenetanshinquinone	C18 H14 O3	✓	x	x	x	
Isoallylbenzene	**C_{9} H_{10}**	✓	✓	✓	✓	**Hydrocarbures**
1-Propenyl-cyclohexane	C_{9} H_{16}	x	✓	x	✓	

Exaltolide	$C_{15}H_{28}O_2$	✓	x	x	x	**Macrolide**
Adenosine	$C_{10}H_{13}N_5O_4$	✓	✓	✓	✓	**Nucleoside**
Muricatacin	$C_{17}H_{32}O_3$	✓	✓	x	✓	**Acetogenin**
(2S)-1-O-(9Z,12Z-Octadeca-dien-noyl)-3-O-beta-D-galactopyranosyl-glycerol	$C_{27}H_{48}O_9$	✓	x	x	x	**Glycerolipid**
Suaveolol	$C_{20}H_{34}O_2$	✓	x	x	x	**Polycyclic compound**
Wilforonide	$C_{13}H_{16}O_3$	x	x	x	✓	**Keto-ester**
Deoxymorellin	$C_{33}H_{38}O_6$	✓	✓	x	✓	**Miscellenous**
Yonogenin	$C_{27}H_{44}O_4$	✓	x	x	✓	**Saponin**
Urushiol III	$C_{21}H_{32}O_2$	x	✓	x	✓	**Cathecol**
3beta-Methoxy-9beta,19-cyclolanost-23(E)-en-25,26-diol	$C_{31}H_{52}O_3$	x	✓	x	✓	**Vitamin (Vit E)**
8-Methyl-5-isopropyl-6,8-nonadiene-2-one	$C_{12}H_{22}O$	x	x	x	✓	**Ether**
6-Phenylundecane	$C_{17}H_{28}$	x	x	x	✓	**Alkylbenzene**
Sandaracopimarinol	$C_{20}H_{32}O$	x	x	x	✓	**Phenanthrene**
5-Methoxy-1,7-diphenyl-3-heptanone	$C_{20}H_{24}O_2$	x	x	x	✓	**diarylheptanoid**
Spatheliabischromene	$C_{20}H_{20}O_4$	✓	x	x	x	**Benzopyranoid**
Icaride A2	$C_{22}H_{28}O_9$	✓	x	x	x	**Phenylpropanoid**
Margaspidin	$C_{24}H_{30}O_8$	✓	x	x	x	**Phloroglucinol derivative**
Shikonofuran C	$C_{21}H_{26}O_5$	✓	x	x	x	**Shikonin derivative**

Identification of isolated compound

The structure of the compound CF_{16} was determined on the basis of spectral data. This structure was confirmed by comparing with those described in literature (Moradkhani*et al.,* 2014). By comparison of the data with those reported in the literature, the compound was identified as Daucosterol (β- sitosterol 3-O- β- D- glucopyranoside) (Fig. 1). It has a molecular weight of 576g/mol, corresponding to the empirical formula $C_{35}H_{60}O_6$.

The isolated compound was found to be inactive on all the tested bacteria. These results are similar to those reported by Bayor *et al.,* (2009) who evaluated β-sitosterol-3-O-D-glucopyranoside on *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and it had no antibacterial effect. Njinga *et al.,* (2016) reported a good activity of the molecule on a set of bacteria including *S.aureus* and *E.coli*, with MIC ranged from 25 to 50 µg/ml. This different result could be due to the fact that Njinga *et al.,* used just a loop of a 0,5 McFarland bacterial solution as the inoculum; this is very low comparing to that we used in our work.

HPLC-MS analysis

The chemical analysis of the F_6 fraction, the sub-fractions F'_3,F'_4 and F'_5revealed the presence of almost 180 identified compounds from various classes of secondary metabolites including alkaloids, flavonoids, phenols, quinones, steroids and terpenoids (Table 2). These phytochemicals may explain their antibacterial capacity, since the inhibitory properties of these secondary metabolites against different pathogens have been reported (Cowan, 1999). For example, flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins. More lipophilic flavonoids disrupt cell wall integrity (Kurtz *et al.,* 1994). The chemical analysis revealed that many compounds that had not been detected in the F_6 fraction were found in the sub-fractions, suggesting that their concentration increased during the fractionation process. This may explain the increased antibacterial ability of sub-fractions F'_3, F'_4 and F'_5 compared to the F_6 fraction.

In conclusion, the results revealed an increased activity with fractionation, the sub-fractions F'_4 and F'_5 being the most active. These sub-fractions could be used as sources of antibacterial compounds.

References

Aboudi, E.C.E., Mouokeu, R.S., Njateng, G.S.S., Tchinda, T.A., Etame, R.E. Rosalie Anne Ngono, N.A.R. 2019.Antibacterial Activity of Methanol Extract and Fractions from Stem Bark of *Bridelia micrantha* (Hochst.)Baill. (*Phyllanthaceae*). *EC Pharmacology and Toxicology*. 7(7): 609-616.

Adefuye, A. and Ndip, R. N. 2013. Phytochemical analysis and antibacterial evaluation of the ethyl acetate extract of the stem bark of *Brideliamicrantha*. *Pharmacognosy Magazine*. 9: 45–50.

Adefuye, A.O., Samie, A., Ndip, R.N. 2011. "*In Vitro* evaluation of the antimicrobial activity of extracts of *Bridelia micrantha* on selected bacterial pathogens". *Journal of Medicinal Plants Research*. 5: 5116–5122.

Algiannis, N., Kalpotzakis, E., Mitaku, S., Chinou, I.B. 2001. Composition and antimicrobial activity of the essential oils of two *Origanum* species. *Journal of Agricultural and Food Chemistry*. 40: 4168-4170.

Bayor, M.T., Gbedema, S.Y., Anna, K. 2009. The antimicrobial activity of *Croton membranaceus*, a species used in

formulations for measles in Ghana. *J Pharmacogn Phytother*. 1:47-51.

Clinical and Laboratory Standard Institute (CLSI). 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically approved standard. *9th edition*, 35(2):1-15.

Cosgrove, S.E., Sakoulas, G., Perencevich, E.N., Schwaber, M.J., Karchmer, A.W., Carmeli, Y. 2003. Comparison of mortality associated with methicillin-resistant and methicillin susceptible *Staphylococcus aureus* bacteremia meta- analysis. *Clin. Infect. Dis*. 36: 53-59.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 12: 564–582.

Djeussi, E., Noumedem, A.K., Seukep, J.A., Fankam, A.G., Voukeng, K.I., Tankeo S.B., Nkuete, H.L., Kuete, V.2013. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complementary and Alternative Medicine*. 13: 164.

Etame, R.E., Mouokeu, R.S., Pouaha, C.L.C., Kenfack, I. V., Tchientcheu, R., Assam, J.P.A., Monthe, P. F.S., Tchinda, A.T., Etoa, F.X., Kuiate, J.R., Ngono, N.R.A. 2018. Effect of Fractioning on Antibacterial Activity of *Enantia chlorantha* Oliver (Annonaceae) Methanol Extract and Mode of Action. *Evidence-Based Complementary and Alternative Medicine*. Article ID 4831593, 13 pages.

Etame, R.M.E., Mouokeu, R.S., Monthe, P.F.S., Voukeng, K.I., Cidjeu, P.L.C., Tchinda, T.A., Yaya, G.A.J., Ngono, N.A.R, Kuiate, R.J., Etoa, F.X. 2019.Effect of fractioning on antibacterial activity of n-butanol fraction from *Enantia chlorantha* stem bark methanol extract. *BMC Complementary and Alternative Medicine*.19:56

Jean, A.A., Robert, P.B., Quirico, J., Nelson, Z., Giselle, T., Guy, H.H. 2001. Separation of crude plant extracts with high speed CCC for primary screening in drug discovery. *J. Liquid Chromatogr. Related Technol.* 24 (11 & 12): 1827-1840.

Gangoué-Piéboji, J., Eze, N., Djintchui, A.N., Ngameni, B., Tsabang, N., Pegnyemb, D.E., Biyiti, L., Ngassam, P., Koulla-Shiro, S., Galleni, M. 2009. The in vitro antimicrobial activity of some traditionally used medicinal plants against beta-lactam-resistant bacteria. *J. Infect. Dev. Ctries*. 3: 671–680.

Groupe National de Guidance en matière de Prévention des Infections Nosocomiales (GNPIN). 2018. Recommandations Nationales pour la lutte contre le *Staphylococcus Aureus* Résistant à la Méticilline (Methicilline Resistant *Staphylococcus aureus*) dans les établissements hospitaliers. Luxembourg.

Hamamoto, H., Urai, M., Ishii, K., Yasukawa, J., Paudel, A., Murai, M., Kaji, T., Kuranaga, T., Hamase, K., Katsu, T., Su, J., Adachi, T., Uchida, R., Tomoda, H., Yamada, M., Souma, M., Kurihara, H., Inoue, M., Sekimizu, K. 2015. Lysocin E is a new antibiotic that targets menaquinone in the bacterial membrane. *Nature Chemical Biology*. 11(2): 127-133.

Holley, R. and Patel, D. 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*. 22: 273-292.

Hussein, R. A., El-Anssary, A. A. 2018. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants.

Khan, M.A.A., Islam, M.T., Rahman, M. A., Ahsan, Q. 2011. Antibacterial activity

of diff erent fractions of *Commelina benghalensis* L. *Der Pharmacia Sinica, Pelagia Research Library*. 2(2): 320–326.

Kharma, A. and Hassawi, D. S. 2006: The Genetic Relationship and Antimicrobial Activity of Plantago species Against Pathogenic Bacteria. *World Journal of Agricultural Sciences*. 2(3): 311-318.

Kuete, V., Fozing, D.C., Kapche W.F., Mbaveng, A.T., Keumedjio, F., Ngadjui, B.T. 2009. Antimicrobial activity of the methanolic extract and compounds from *Morus mesozygia* stem bark. *Journal of Ethnopharmacology*. 124: 551-555.

Kurtz, M.B., Heath, I.B., Marrinan, J., Dreikhorn, S., Onishi, J., Douglas, C. 1994. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-beta-D-glucan synthase. *Antimicrob Agents Chem* 38(7): 1480-1489.

Maroyi, A., 2017. Ethnopharmacology and Therapeutic Value of *Bridelia micrantha* (Hochst.) Baill. in Tropical Africa: A Comprehensive Review. *Molecules,* 22(9).

Montanari, B. 2012. A critical analysis of the introduction of essential oil distillation in the High Atlas of Morocco with reference to the role of gendered traditional knowledge (PhD thesis) *University of Kent, UK: School of Anthropology and conservation*.

Moradkhani, S., Kobarfard, F., Ayatollahi, S.A.M. 2014. Phytochemical Investigations on Chemical Constituents of *Achillea tenuifolia* Lam. *Iranian Journal of Pharmaceutical Research*, 13(3): 1049-1054.

Mouokeu, R.S., Njateng, G.S., Ngono, N.A., Kuiate, J.R., Kamtchueng, M.O. 2014. Antifungal and antioxidant activity of *Crassocephalum bauchiense* (Hutch.) Milne-Redh ethyl acetate extract and fractions (Asteraceae). *BMC Research Notes*.7: 244.

Mouokeu, R. S., Ngono, R. A. N., Lunga, P. K., Koanga, M. M., Tiabou, A. T., Njateng, G.S.S., Tamokou, J. D.D., Kuiate, J-R. 2011. Antibacterial and dermal toxicological profles of ethyl acetate extract from *Crassocephalum bauchiense* (Hutch.) Milne-Redh (Asteraceae). *BMC Complementary and Alternative Medicine.*11(43).

Namita, P. and Mukesh, R. 2012. Medicinal plants used as antimicrobial agents: a review. *Int. Res. J. Pharm*. 3: 31–40.

Ngono, N. R. A., Koanga, M. M. L., Tchinda, T. A., Magnifouet, N. H., Motso, C. P. R., Mballa, B. Z., Ebelle, E. R. M., Ndifor, F., Biyiti, L., Amvam, Z. P. H. 2011. Ethnobotanical survey of some Cameroonian plants used for treatment of viral diseases. *African Journal of Plant Science*. 5(1): 15-21.

Ngueyem, T.A., Brusotti, G., Caccialanza, G., Finzi, P.V. 2009. The genus *Bridelia*: A phytochemical and ethnopharmacological review. *J. Ethnopharmacol.*12: 339–349.

Njinga, N.S., Sule, M.I., Pateh, U.U., Hassan H.S., Abdullahi, S.T.,Ache, R.N. 2016. Isolation and Antimicrobial Activity of β-Sitosterol-3-OGlucoside from *Lannea Kerstingii* Engl. & K. Krause (Anacardiacea). *Nitte University Journal of Health Science*. 6(1).

Nwodo, U.U., Ngene, A.A., Iroegbu, C.U. 2010.Effects of Fractionation on Antibacterial Activity of Crude Extracts of *Tamarindus indica*. *Afr. J. Biotechnol.* 9: 7108-7113.

Peterson, L.R. 2009. Bad bugs, no drugs: no ESCAPE revisited. *Clin Infect Dis*. 49: 992.

Steenkamp, V., A.C. Fernandes and C.E. van Rensburg, 2007. Antibacterial activity of Venda medicinal plants. *Fitoterapia,*

78: 561–564.

Terra, L., Dyson, P.J., Hitchings, M.D., Thomas, L., Abdelhameed, A., Banat, I.M., Gazze, S.A., Vujaklija, D., Facey, P.D., Francis, L.W., Quinn, G.A. 2018. A Novel Alkaliphilic Streptomyces Inhibits ESKAPE Pathogens. *Frontiers in Microbiology*.

Umar, Z., Qureshi, A.S., Usman, M., Sarfraz, A., Umar, S., Umar, T., Hussain, M. 2018. Response of dietary supplementation of black seed (Nigella sativa) oil on hematological parameters, serum biochemistry and reproductive hormones in male rabbits. *Academia Publishing*. 6(9): 276-280.

Verpoorte, R. 1998. Exploration of nature's chemodiversity: The role of secondary metabolites as leads in drug development. *Drug Dev. Trends*. 3: 232-233.

World Health Organization (WHO).2014. Antimicrobial Resistance. *Global Report on Surveillance.*

How to cite this article:

Colette ElyseeAboudi Etono, Raymond Simplice Mouokeu, Guy SedarSingor Njateng, Alembert Tchinda Tiabou, Rebecca Madeleine Ebelle Etame, Rosalie Anne Ngono Ngane and MouelleSone Albert. 2020. Chemical Composition and Antibacterial Activity of Fractions from Bridelia micrantha Stem Bark Methanol Extract. *Int.J.Curr.Microbiol.App.Sci.* 9(01): 1102-1119. doi: https://doi.org/10.20546/ijcmas.2020.901.125